AMENDMENTS TO THE SPECIFICATION:

Please replace paragraph [005] with the following amended paragraph marked to show changes:

[005] The Fc region of an antibody interacts with a number of Fc receptors and ligands, imparting an array of important functional capabilities referred to as effector functions. For IgG the Fc region, as shown in Figure 1, comprises Ig domains Cγ2 and Cγ3 and the N-terminal hinge leading into Cγ2. An important family of Fc receptors for the IgG class are the Fc gamma receptors (FcγRs). These receptors mediate communication between antibodies and the cellular arm of the immune system (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ravetch *et al.*, 2001, *Annu Rev Immunol* 19:275-290). In humans this protein family includes FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2) (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65). These receptors typically have an extracellular domain that mediates binding to Fc, a membrane spanning region, and an intracellular domain that may mediate some signaling event within the cell. These receptors are expressed in a variety of immune cells including monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, and γγ T cells. Formation of the Fc/FcγR complex recruits these effector cells to sites of bound antigen, typically resulting in signaling events within the cells and important subsequent immune responses such as release of inflammation mediators, B cell activation, endocytosis, phagocytosis, and cytotoxic attack. The ability to mediate cytotoxic and phagocytic effector functions is a potential mechanism by which antibodies destroy targeted cells. The cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell is referred to as antibody dependent cell-mediated cytotoxicity (ADCC) (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ghetie *et al.*, 2000, *Annu Rev Immunol* 18:739-766; Ravetch *et al.*, 2001, *Annu Rev Immunol* 19:275-290). The cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the

target cell is referred to as antibody dependent cell-mediated phagocytosis (ADCP). A number of structures have been solved of the extracellular domains of human FcγRs, including FcγRIIa (pdb accession code 1H9V)(Sondermann *et al.*, 2001, *J Mol Biol* 309:737-749) (pdb accession code 1FCG)(Maxwell *et al.*, 1999, *Nat Struct Biol* 6:437-442), FcγRIIb (pdb accession code 2FCB)(Sondermann *et al.*, 1999, *Embo J* 18:1095-1103); and FcγRIIIb (pdb accession code 1E4J)(Sondermann *et al.*, 2000, *Nature* 406:267-273.). All FcγRs bind the same region on Fc, at the N-terminal end of the Cγ2 domain and the preceding hinge, shown in Figure 2. This interaction is well characterized structurally (Sondermann *et al.*, 2001, *J Mol Biol* 309:737-749), and several structures of the human Fc bound to the extracellular domain of human FcγRIIIb have been solved (pdb accession code 1E4K)(Sondermann *et al.*, 2000, *Nature* 406:267-273.) (pdb accession codes 1IIS and 1IIX)(Radaev *et al.*, 2001, *J Biol Chem* 276:16469-16477), as well as has the structure of the human IgE ~~Fc/Fc□RI□~~ Fc/FcεRIα complex (pdb accession code 1F6A)(Garman *et al.*, 2000, *Nature* 406:259-266).

Please replace paragraph [044] with the following amended paragraph marked to show changes:

[044] Figure 9. Alemtuzumab expressed from 293T cells binds its antigen. The antigenic CD52 peptide, fused to GST, was expressed in *E. coli* BL21 (DE3) under IPTG induction. Both uninduced and induced samples were run on a SDS-PAGE gel, and transferred to PVDF membrane. For western analysis, either alemtuzumab from Sotec (~~□CD52~~ α-CD52, Sotec) (final concentration 2.5ng/ul) or media of transfected 293T cells (Campath, Xencor) (final alemtuzumab concentration approximately 0.1-0.2ng/ul) were used as primary antibody, and peroxidase-conjugated goat-anti human IgG was used as secondary antibody. M: pre-stained marker; U: un-induced sample for GST-CD52; I: induced sample for GST-CD52.

Please replace paragraph [071] with the following amended paragraph marked to show changes:

[071] By "antibody" herein is meant a protein consisting of one or more polypeptides substantially encoded by all or part of the recognized immunoglobulin genes. The recognized immunoglobulin genes, for example in humans, include the kappa (□κ), lambda (□λ), and heavy chain genetic loci, which together comprise the myriad variable region genes, and the constant region genes mu (□μ), delta (□δ), gamma (γ), sigma (□σ), and alpha (□α) which

encode the IgM, IgD, IgG, IgE, and IgA isotypes respectively. Antibody herein is meant to include full length antibodies and antibody fragments, and may refer to a natural antibody from any organism, an engineered antibody, or an antibody generated recombinantly for experimental, therapeutic, or other purposes as further defined below. Thus, "antibody" includes both polyclonal and monoclonal antibody (mAb). Methods of preparation and purification of monoclonal and polyclonal antibodies are known in the art and e.g., are described in Harlow and Lane, Antibodies: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1988). As outlined herein, "antibody" specifically includes Fc variants described herein, "full length" antibodies including the Fc variant fragments described herein, and Fc variant fusions to other proteins as described herein.

Please replace paragraph [086] with the following amended paragraph marked to show changes:

[086] By "IgG" as used herein is meant a polypeptide belonging to the class of antibodies that are substantially encoded by a recognized immunoglobulin gamma gene. In humans this class comprises IgG1, IgG2, IgG3, and IgG4. In mice this class comprises IgG1, IgG2a, IgG2b, IgG3. By "immunoglobulin (Ig)" herein is meant a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. Immunoglobulins include but are not limited to antibodies. Immunoglobulins may have a number of structural forms, including but not limited to full length antibodies, antibody fragments, and individual immunoglobulin domains. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin that exists as a distinct structural entity as ascertained by one skilled in the art of protein structure. Ig domains typically have a characteristic β-sandwich folding topology. The known Ig domains in the IgG class of antibodies are V_H, Cγ1, Cγ2, Cγ3, V_L, and C_L.

Please replace paragraph [092] with the following amended paragraph marked to show changes:

[092] By "variable region" as used herein is meant the region of an immunoglobulin that comprises one or more Ig domains substantially encoded by any of the ~~V~~Vκ, ~~V~~Vλ, and/or V_H genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

Please replace paragraph [098] with the following amended paragraph marked to show changes:

[098] The Fc variants of the present invention may be combined with other Fc modifications, including but not limited to modifications that alter effector function. Such combination may provide additive, synergistic, or novel properties in antibodies or Fc fusions. In one embodiment, the Fc variants of the present invention may be combined with other known Fc variants (Duncan *et al.*, 1988, *Nature* 332:563-564; Lund *et al.*, 1991, *J Immunol* 147:2657-2662; Lund *et al.*, 1992, *Mol Immunol* 29:53-59; Alegre *et al.*, 1994, *Transplantation* 57:1537-1543; Hutchins *et al.*, 1995, *Proc Natl Acad Sci U S A* 92:11980-11984; Jefferis *et al.*, 1995, *Immunol Lett* 44:111-117; Lund *et al.*, 1995, *Faseb J* 9:115-119; Jefferis *et al.*, 1996, *Immunol Lett* 54:101-104; Lund *et al.*, 1996, *J Immunol* 157:4963-4969; Armour *et al.*, 1999, *Eur J Immunol* 29:2613-2624; Idusogie *et al.*, 2000, *J Immunol* 164:4178-4184; Reddy *et al.*, 2000, *J Immunol* 164:1925-1933; Xu *et al.*, 2000, *Cell Immunol* 200:16-26; Idusogie *et al.*, 2001, *J Immunol* 166:2571-2575; Shields *et al.*, 2001, *J Biol Chem* 276:6591-6604; Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65; Presta *et al.*, 2002, *Biochem Soc Trans* 30:487-490) (US 5,624,821; US 5,885,573; US 6,194,551; PCT WO 00/42072; PCT WO 99/58572). In an alternate embodiment, the Fc variants of the present invention are incorporated into an antibody or Fc fusion that comprises one or more engineered glycoforms. By "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to an Fc polypeptide, wherein said carbohydrate composition differs chemically from that of a parent Fc polypeptide. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. Engineered glycoforms may be generated by any method, for example by using engineered or variant expression strains, by co-expression with one or more enzymes, for example ⊟β1–4- N-acetylglucosaminyltransferase III (GnTIII), by expressing an Fc polypeptide in various organisms or cell lines from various organisms, or by modifying carbohydrate(s) after the Fc polypeptide has been expressed. Methods for generating engineered glycoforms are known in the art, and include but are not limited to (Umaña *et al.*, 1999, *Nat Biotechnol* 17:176-180; Davies *et al.*, 2001, *Biotechnol Bioeng* 74:288-294; Shields *et al.*, 2002, *J Biol Chem* 277:26733-26740; Shinkawa *et al.*, 2003, *J Biol Chem* 278:3466-3473) US 6,602,684; USSN 10/277,370; USSN 10/113,929; PCT WO 00/61739A1; PCT WO 01/29246A1; PCT WO 02/31140A1; PCT WO 02/30954A1; Potelligent™ technology (Biowa, Inc., Princeton, N.J.); GlycoMAb™ glycosylation

engineering technology (GLYCART biotechnology AG, Zürich, Switzerland)). Engineered glycoform typically refers to the different carbohydrate or oligosaccharide; thus an Fc polypeptide, for example an antibody or Fc fusion, may comprise an engineered glycoform. Alternatively, engineered glycoform may refer to the Fc polypeptide that comprises the different carbohydrate or oligosaccharide. Thus combinations of the Fc variants of the present invention with other Fc modifications, as well as undiscovered Fc modifications, are contemplated with the goal of generating novel antibodies or Fc fusions with optimized properties.

Please replace paragraph [101] with the following amended paragraph marked to show changes:

[101] One skilled in the art will appreciate that the aforementioned list of targets refers not only to specific proteins and biomolecules, but the biochemical pathway or pathways that comprise them. For example, reference to CTLA-4 as a target antigen implies that the ligands and receptors that make up the T cell co-stimulatory pathway, including CTLA-4, B7-1, B7-2, CD28, and any other undiscovered ligands or receptors that bind these proteins, are also targets. Thus target as used herein refers not only to a specific biomolecule, but the set of proteins that interact with said target and the members of the biochemical pathway to which said target belongs. One skilled in the art will further appreciate that any of the aforementioned target antigens, the ligands or receptors that bind them, or other members of their corresponding biochemical pathway, may be operably linked to the Fc variants of the present invention in order to generate an Fc fusion. Thus for example, an Fc fusion that targets EGFR could be constructed by operably linking an Fc variant to EGF, ~~TGF□~~ TGFα, or any other ligand, discovered or undiscovered, that binds EGFR. Accordingly, an Fc variant of the present invention could be operably linked to EGFR in order to generate an Fc fusion that binds EGF, ~~TGF□~~ TGFα, or any other ligand, discovered or undiscovered, that binds EGFR. Thus virtually any polypeptide, whether a ligand, receptor, or some other protein or protein domain, including but not limited to the aforementioned targets and the proteins that compose their corresponding biochemical pathways, may be operably linked to the Fc variants of the present invention to develop an Fc fusion.

Please replace paragraph [102] with the following amended paragraph marked to show changes:

[102] A number of antibodies and Fc fusions that are approved for use, in clinical trials, or in development may benefit from the Fc variants of the present invention. Said antibodies and Fc fusions are herein referred to as "clinical products and candidates". Thus in a preferred embodiment, the Fc variants of the present invention may find use in a range of clinical products and candidates. For example, a number of antibodies that target CD20 may benefit from the Fc variants of the present invention. For example the Fc variants of the present invention may find use in an antibody that is substantially similar to rituximab (Rituxan®, IDEC/Genentech/Roche) (see for example US 5,736,137), a chimeric anti-CD20 antibody approved to treat Non-Hodgkin's lymphoma; HuMax-CD20, an anti-CD20 currently being developed by Genmab, an anti-CD20 antibody described in US 5,500,362, AME-133 (Applied Molecular Evolution), hA20 (Immunomedics, Inc.), and HumaLYM (Intracel). A number of antibodies that target members of the family of epidermal growth factor receptors, including EGFR (ErbB-1), Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), may benefit from the Fc variants of the present invention. For example the Fc variants of the present invention may find use in an antibody that is substantially similar to trastuzumab (Herceptin®, Genentech) (see for example US 5,677,171), a humanized anti-Her2/neu antibody approved to treat breast cancer; pertuzumab (rhuMab-2C4, Omnitarg™), currently being developed by Genentech; an anti-Her2 antibody described in US 4,753,894; cetuximab (Erbitux®, Imclone) (US 4,943,533; PCT WO 96/40210), a chimeric anti-EGFR antibody in clinical trials for a variety of cancers; ABX-EGF (US 6,235,883), currently being developed by Abgenix/Immunex/Amgen; HuMax-EGFr (USSN 10/172,317), currently being developed by Genmab; 425, EMD55900, EMD62000, and EMD72000 (Merck KGaA) (US 5558864; Murthy et al. 1987, *Arch Biochem Biophys.* 252(2):549-60; Rodeck et al., 1987, *J Cell Biochem.* 35(4):315-20; Kettleborough et al., 1991, *Protein Eng.* 4(7):773-83); ICR62 (Institute of Cancer Research) (PCT WO 95/20045; Modjtahedi et al., 1993, *J. Cell Biophys.* 1993, 22(1-3):129-46; Modjtahedi et al., 1993, *Br J Cancer.* 1993, 67(2):247-53; Modjtahedi et al, 1996, *Br J Cancer*, 73(2):228-35; Modjtahedi et al, 2003, *Int J Cancer,* 105(2):273-80); TheraCIM hR3 (YM Biosciences, Canada and Centro de Immunologia Molecular, Cuba (US 5,891,996; US 6, 506,883; Mateo et al, 1997, *Immunotechnology*, 3(1):71-81); mAb-806 (Ludwig Institue for Cancer Research, Memorial Sloan-Kettering) (Jungbluth et al. 2003, *Proc Natl Acad Sci U S A.* 100(2):639-44); KSB-102 (KS Biomedix); MR1-1 (IVAX, National Cancer Institute) (PCT WO 0162931A2); and SC100 (Scancell) (PCT WO 01/88138). In another preferred embodiment, the Fc variants of the present invention may

find use in alemtuzumab (Campath®, Millenium), a humanized monoclonal antibody currently approved for treatment of B-cell chronic lymphocytic leukemia. The Fc variants of the present invention may find use in a variety of antibodies or Fc fusions that are substantially similar to other clinical products and candidates, including but not limited to muromonab-CD3 (Orthoclone OKT3®), an anti-CD3 antibody developed by Ortho Biotech/Johnson & Johnson, ibritumomab tiuxetan (Zevalin®), an anti-CD20 antibody developed by IDEC/Schering AG, gemtuzumab ozogamicin (Mylotarg®), an anti-CD33 (p67 protein) antibody developed by Celltech/Wyeth, alefacept (Amevive®), an anti-LFA-3 Fc fusion developed by Biogen), abciximab (ReoPro®), developed by Centocor/Lilly, basiliximab (Simulect®), developed by Novartis, palivizumab (Synagis®), developed by MedImmune, infliximab (Remicade®), an anti-TNFalpha antibody developed by Centocor, adalimumab (Humira®, an anti-TNFalpha antibody developed by Abbott, Humicade™, an anti-TNFalpha antibody developed by Celltech, etanercept (Enbrel®), an anti-TNFalpha Fc fusion developed by Immunex/Amgen, ABX-CBL, an anti-CD147 antibody being developed by Abgenix, ABX-IL8, an anti-IL8 antibody being developed by Abgenix, ABX-MA1, an anti-MUC18 antibody being developed by Abgenix, Pemtumomab (R1549, ^{90}Y-muHMFG1), an anti-MUC1 In development by Antisoma, Therex (R1550), an anti-MUC1 antibody being developed by Antisoma, AngioMab (AS1405), being developed by Antisoma, HuBC-1, being developed by Antisoma, Thioplatin (AS1407) being developed by Antisoma, Antegren® (natalizumab), an anti-alpha-4-beta-1 (VLA-4) and alpha-4-beta-7 antibody being developed by Biogen, VLA-1 mAb, an anti-VLA-1 integrin antibody being developed by Biogen, LTBR mAb, an anti-lymphotoxin beta receptor (LTBR) antibody being developed by Biogen, CAT-152, an ~~anti-TGF□2~~ anti-TGFβ2 antibody being developed by Cambridge Antibody Technology, J695, an anti-IL-12 antibody being developed by Cambridge Antibody Technology and Abbott, CAT-192, an ~~anti-TGF□1~~anti-TGFβ1 antibody being developed by Cambridge Antibody Technology and Genzyme, CAT-213, an anti-Eotaxin1 antibody being developed by Cambridge Antibody Technology, LymphoStat-B™ an anti-Blys antibody being developed by Cambridge Antibody Technology and Human Genome Sciences Inc., TRAIL-R1mAb, an anti-TRAIL-R1 antibody being developed by Cambridge Antibody Technology and Human Genome Sciences, Inc., Avastin™ (bevacizumab, rhuMAb-VEGF), an anti-VEGF antibody being developed by Genentech, an anti-HER receptor family antibody being developed by Genentech, Anti-Tissue Factor (ATF), an anti-Tissue Factor antibody being developed by Genentech, Xolair™ (Omalizumab), an anti-IgE antibody being

developed by Genentech, Raptiva™ (Efalizumab), an anti-CD11a antibody being developed by Genentech and Xoma, MLN-02 Antibody (formerly LDP-02), being developed by Genentech and Millenium Pharmaceuticals, HuMax CD4, an anti-CD4 antibody being developed by Genmab, HuMax-IL15, an anti-IL15 antibody being developed by Genmab and Amgen, HuMax-Inflam, being developed by Genmab and Medarex, HuMax-Cancer, an anti-Heparanase I antibody being developed by Genmab and Medarex and Oxford GcoSciences, HuMax-Lymphoma, being developed by Genmab and Amgen, HuMax-TAC, being developed by Genmab, IDEC-131, and anti-CD40L antibody being developed by IDEC Pharmaceuticals, IDEC-151 (Clenoliximab), an anti-CD4 antibody being developed by IDEC Pharmaceuticals, IDEC-114, an anti-CD80 antibody being developed by IDEC Pharmaceuticals, IDEC-152, an anti-CD23 being developed by IDEC Pharmaceuticals, anti-macrophage migration factor (MIF) antibodies being developed by IDEC Pharmaceuticals, BEC2, an anti-idiotypic antibody being developed by Imclone, IMC-1C11, an anti-KDR antibody being developed by Imclone, DC101, an anti-flk-1 antibody being developed by Imclone, anti-VE cadherin antibodies being developed by Imclone, CEA-Cide™ (labetuzumab), an anti-carcinoembryonic antigen (CEA) antibody being developed by Immunomedics, LymphoCide™ (Epratuzumab), an anti-CD22 antibody being developed by Immunomedics, AFP-Cide, being developed by Immunomedics, MyelomaCide, being developed by Immunomedics, LkoCide, being developed by Immunomedics, ProstaCide, being developed by Immunomedics, MDX-010, an anti-CTLA4 antibody being developed by Medarex, MDX-060, an anti-CD30 antibody being developed by Medarex, MDX-070 being developed by Medarex, MDX-018 being developed by Medarex, Osidem™ (IDM-1), and anti-Her2 antibody being developed by Medarex and Immuno-Designed Molecules, HuMax™-CD4, an anti-CD4 antibody being developed by Medarex and Genmab, HuMax-IL15, an anti-IL15 antibody being developed by Medarex and Genmab, CNTO 148, an ~~anti-TNF□~~ anti-TNFα antibody being developed by Medarex and Centocor/J&J, CNTO 1275, an anti-cytokine antibody being developed by Centocor/J&J, MOR101 and MOR102, anti-intercellular adhesion molecule-1 (ICAM-1) (CD54) antibodies being developed by MorphoSys, MOR201, an anti-fibroblast growth factor receptor 3 (FGFR-3) antibody being developed by MorphoSys, Nuvion® (visilizumab), an anti-CD3 antibody being developed by Protein Design Labs, HuZAF™, an anti-gamma interferon antibody being developed by Protein Design Labs, ~~Anti-□5□1~~ Anti-α5β1 Integrin, being developed by Protein Design Labs, anti-IL-12, being developed by Protein Design Labs, ING-1, an anti-Ep-CAM antibody

being developed by Xoma, and MLN01, an anti-Beta2 integrin antibody being developed by Xoma.

Please replace paragraph [114] with the following amended paragraph marked to show changes:

[114] Chemotherapeutic agents that may be useful for conjugation to the antibodies and Fc fusions of the present invention have been described above. In an alternate embodiment, the antibody or Fc fusion is conjugated or operably linked to a toxin, including but not limited to small molecule toxins and enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Small molecule toxins include but are not limited to calicheamicin, maytansine (US 5,208,020), trichothene, and CC1065. In one embodiment of the invention, the antibody or Fc fusion is conjugated to one or more maytansine molecules (e.g. about 1 to about 10 maytansine molecules per antibody molecule). Maytansine may, for example, be converted to May-SS-Me which may be reduced to May-SH3 and reacted with modified antibody or Fc fusion (Chari *et al.*, 1992, *Cancer Research* 52: 127-131) to generate a maytansinoid-antibody or maytansinoid-Fc fusion conjugate. Another conjugate of interest comprises an antibody or Fc fusion conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin that may be used include but are not limited to γ_1^1, α_2^1, α_3, N-acetyl-γ_1^1, PSAG, and $\theta^1{}_1$, (Hinman *et al.*, 1993, *Cancer Research* 53:3336-3342; Lode *et al.*, 1998, *Cancer Research* 58:2925-2928) (US 5,714,586; US 5,712,374; US 5,264,586; US 5,773,001). Dolastatin 10 analogs such as auristatin E (AE) and monomethylauristatin E (MMAE) may find use as conjugates for the Fc variants of the present invention (Doronina *et al.*, 2003, *Nat Biotechnol* 21(7):778-84; Francisco *et al.*, 2003 *Blood* 102(4):1458-65). Useful enyzmatically active toxins include but are not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, PCT WO 93/21232. The present invention further contemplates a conjugate or fusion formed between an antibody or Fc fusion of the present invention and a compound

with nucleolytic activity, for example a ribonuclease or DNA endonuclease such as a deoxyribonuclease (DNase).

Please replace paragraph [128] with the following amended paragraph marked to show changes:

[128] An additional design strategy for engineering Fc variants is provided in which the conformation of the Cγ2 domain is optimized Optimization as used in this context is meant to describe conformational changes in the Cγ2 domain angle that result in a desired property, for example increased or reduced affinity for an FcγR. By exploring energetically favorable substitutions at Cγ2 positions that impact the Cγ2 conformation, a quality diversity of variants can be engineered that sample new Cγ2 conformations, some of which may achieve the design goal. Such new Cγ2 conformations could be the result of, for example, alternate backbone conformations that are sampled by the variant. Variable positions may be chosen as any positions that are believed to play an important role in determining ~~C□2~~ Cγ2 structure, stability, solubility, flexibility, function, and the like. For example, Cγ2 hydrophobic core residues, that is Cγ2 residues that are partially or fully sequestered from solvent, may be reengineered. Alternatively, noncore residues may be considered, or residues that are deemed important for determining backbone structure, stability, or flexibility.

Please replace paragraph [136] with the following amended paragraph marked to show changes:

[136] Once a template structure has been obtained, variable positions are chosen. By "variable position" herein is meant a position at which the amino acid identity is allowed to be altered in a computational screening calculation. As is known in the art, allowing amino acid modifications to be considered only at certain variable positions reduces the complexity of a calculation and enables computational screening to be more directly tailored for the design goal. One or more residues may be variable positions in computational screening calculations. Positions that are chosen as variable positions may be those that contribute to or are hypothesized to contribute to the protein property to be optimized, for example Fc affinity for an FcγR, Fc stability, Fc solubility, and so forth. Residues at variable positions may contribute favorably or unfavorably to a specific protein property. For example, a residue at an Fc/FcγR interface may be involved in mediating binding, and thus this position may be

varied in design calculations aimed at improving Fc/FcγR affinity. As another example, a residue that has an exposed hydrophobic side chain may be responsible for causing unfavorable aggregation, and thus this position may be varied in design calculations aimed at improving solubility. Variable positions may be those positions that are directly involved in interactions that are determinants of a particular protein property. For example, the ~~Fc□R~~ FcγR binding site of Fc may be defined to include all residues that contact that particular FγcR. By "contact" herein is meant some chemical interaction between at least one atom of an Fc residue with at least one atom of the bound FcγR, with chemical interaction including, but not limited to van der Waals interactions, hydrogen bond interactions, electrostatic interactions, and hydrophobic interactions. In an alternative embodiment, variable positions may include those positions that are indirectly involved in a protein property, i.e. such positions may be proximal to residues that are known to or hypothesized to contribute to an Fc property. For example, the FcγR binding site of an Fc may be defined to include all Fc residues within a certain distance, for example 4 - 10 Å, of any Fc residue that is in van der Waals contact with the FcγR. Thus variable positions in this case may be chosen not only as residues that directly contact the FcγR, but also those that contact residues that contact the FcγR and thus influence binding indirectly. The specific positions chosen are dependent on the design strategy being employed.

Please replace paragraph [138] with the following amended paragraph marked to show changes:

[138] Positions that are not variable or floated are fixed. By "fixed position" herein is meant a position at which the amino acid identity and the conformation are held constant in a computational screening calculation. Positions that may be fixed include residues that are not known to be or hypothesized to be involved in the property to be optimized. In this case the assumption is that there is little or nothing to be gained by varying these positions. Positions that are fixed may also include positions whose residues are known or hypothesized to be important for maintaining proper folding, structure, stability, solubility, and/or biological function. For example, positions may be fixed for residues that interact with a particular Fc ligand or residues that encode a glycosylation site in order to ensure that binding to the Fc ligand and proper glycosylation respectively are not perturbed. Likewise, if stability is being optimized, it may be beneficial to fix positions that directly or indirectly interact with an Fc ligand, for example an ~~Fc□R~~FcγR, so that binding is not perturbed. Fixed positions may also

include structurally important residues such as cysteines participating in disulfide bridges, residues critical for determining backbone conformation such as proline or glycine, critical hydrogen bonding residues, and residues that form favorable packing interactions.

Please replace paragraph [140] with the following amended paragraph marked to show changes:

[140] A wide variety of methods may be used, alone or in combination, to select which amino acids will be considered at each position. For example, the set of considered amino acids at a given variable position may be chosen based on the degree of exposure to solvent. Hydrophobic or nonpolar amino acids typically reside in the interior or core of a protein, which are inaccessible or nearly inaccessible to solvent. Thus at variable core positions it may be beneficial to consider only or mostly nonpolar amino acids such as alanine, valine, isoleucine, leucine, phenylalanine, tyrosine, tryptophan, and methionine. Hydrophilic or polar amino acids typically reside on the exterior or surface of proteins, which have a significant degree of solvent accessibility. Thus at variable surface positions it may be beneficial to consider only or mostly polar amino acids such as alanine, serine, threonine, aspartic acid, asparagine, glutamine, glutamic acid, arginine, lysine and histidine. Some positions are partly exposed and partly buried, and are not clearly protein core or surface positions, in a sense serving as boundary residues between core and surface residues. Thus at such variable boundary positions it may be beneficial to consider both nonpolar and polar amino acids such as alanine, serine, threonine, aspartic acid, asparagine, glutamine, glutamic acid, arginine, lysine histidine, valine, isoleucine, leucine, phenylalanine, tyrosine, tryptophan, and methionine. Determination of the degree of solvent exposure at variable positions may be by subjective evaluation or visual inspection of the template structure by one skilled in the art of protein structural biology, or by using a variety of algorithms that are known in the art. Selection of amino acid types to be considered at variable positions may be aided or determined wholly by computational methods, such as calculation of solvent accessible surface area, or using algorithms that assess the orientation of the ~~C□-C□C□~~Cα-Cβ vectors relative to a solvent accessible surface, as outlined in US 6,188,965; 6,269,312; US 6,403,312; USSN 09/782,004; USSN 09/927,790; USSN 10/218,102; PCT WO 98/07254; PCT WO 01/40091; and PCT WO 02/25588. In one embodiment, each variable position may be classified explicitly as a core, surface, or boundary position or a classification substantially similar to core, surface, or boundary.

Please replace paragraph [174] with the following amended paragraph marked to show changes:

[174] In a preferred embodiment, the functional and/or biophysical properties of Fc variants are screened in an *in vitro* assay. *In vitro* assays may allow a broad dynamic range for screening properties of interest. Properties of Fc variants that may be screened include but are not limited to stability, solubility, and affinity for Fc ligands, for example ~~FcRs~~ FcγRs. Multiple properties may be screened simultaneously or individually. Proteins may be purified or unpurified, depending on the requirements of the assay. In one embodiment, the screen is a qualitative or quantitative binding assay for binding of Fc variants to a protein or nonprotein molecule that is known or thought to bind the Fc variant. In a preferred embodiment, the screen is a binding assay for measuring binding to the antibody's or Fc fusions' target antigen. In an alternately preferred embodiment, the screen is an assay for binding of Fc variants to an Fc ligand, including but are not limited to the family of ~~FcRs~~ FcγRs, the neonatal receptor FcRn, the complement protein C1q, and the bacterial proteins A and G. Said Fc ligands may be from any organism, with humans, mice, rats, rabbits, and monkeys preferred. Binding assays can be carried out using a variety of methods known in the art, including but not limited to FRET (Fluorescence Resonance Energy Transfer) and BRET (Bioluminescence Resonance Energy Transfer) -based assays, AlphaScreen™ (Amplified Luminescent Proximity Homogeneous Assay), Scintillation Proximity Assay, ELISA (Enzyme-Linked Immunosorbent Assay), SPR (Surface Plasmon Resonance, also known as BIACORE®), isothermal titration calorimetry, differential scanning calorimetry, gel electrophoresis, and chromatography including gel filtration. These and other methods may take advantage of some fusion partner or label of the Fc variant. Assays may employ a variety of detection methods including but not limited to chromogenic, fluorescent, luminescent, or isotopic labels.

Please replace paragraph [186] with the following amended paragraph marked to show changes:

[186] Several different structures of Fc bound bound to the extracellular domain of ~~FcRs~~ FcγRs served as template structures for the computational screening calculations. Publicly available Fc/FcγR complex structures included pdb accession code 1E4K (Sondermann *et al.*, 2000, *Nature* 406:267-273.), and pdb accession codes 1IIS and 1IIX (Radaev *et al.*, 2001, *J Biol Chem* 276:16469-16477). The extracellular regions of ~~FcRIIIb~~ FcγRIIIb and FcγRIIIa

are 96% identical, and therefore the use of the Fc/FcγRIIIb structure is essentially equivalent to use of FcγRIIIa. Nonetheless, for some calculations, a more precise ~~Fc/Fc□RIIIa~~ Fc/FcγRIIIa template structure was constructed by modeling a D129G mutation in the 1IIS and 1E4K structures (referred to as D129G 1IIS and D129G 1E4K template structures). In addition, the structures for human Fc bound to the extracellular domains of human FcγRIIb, human F158 ~~Fc□RIIIa~~ FcγRIIIa, and mouse FcγRIII were modeled using standard methods, the available ~~Fc□R~~ FcγR sequence information, the aforementioned Fc/FcγR structures, as well as structural information for unbound complexes (pdb accession code 1H9V)(Sondermann *et al.*, 2001, *J Mol Biol* 309:737-749) (pdb accession code 1FCG)(Maxwell *et al.*, 1999, *Nat Struct Biol* 6:437-442), ~~Fc□RIIb~~ FcγRIIb (pdb accession code 2FCB)(Sondermann *et al.*, 1999, *Embo J* 18:1095-1103), and ~~Fc□RIIIb~~ FcγRIIIb (pdb accession code 1E4J)(Sondermann *et al.*, 2000, *Nature* 406:267-273.).

Please replace paragraph [187] with the following amended paragraph marked to show changes:

[187] Variable positions and amino acids to be considered at those positions were chosen by visual inspection of the aforementioned Fc/FcγR and FcγR structures, and using solvent accessibility information and sequence information. Sequence information of Fcs and FcγRs was particularly useful for determining variable positions at which substitutions may provide distinguishing affinities between activating and inhibitory receptors. Virtually all Cγ2 positions were screened computationally. The Fc structure is a homodimer of two heavy chains (labeled chains A and B in the 1IIS, 1IIX, and 1E4K structures) that each include the hinge and ~~C□2-C□3~~ Cγ2-Cγ3 domains (shown in Figure 2). Because the ~~Fc□R~~ FcγR (labeled chain C in the 1IIS, 1IIX, and 1E4K structures) binds asymmetrically to the Fc homodimer, each chain was often considered separately in design calculations. For some calculations, Fc and/or ~~Fc□R~~ FcγR residues proximal to variable position residues were floated, that is the amino acid conformation but not the amino acid identity was allowed to vary in a protein design calculation to allow for conformational adjustments. These are indicated below the table for each set of calculations when relevant. Considered amino acids typically belonged to either the Core, Core XM, Surface, Boundary, Boundary XM, or All 20 classifications, unless noted otherwise. These classifications are defined as follows: Core = alanine, valine, isoleucine, leucine, phenylalanine, tyrosine, tryptophan, and methionine; Core XM = alanine, valine, isoleucine, leucine, phenylalanine, tyrosine, and tryptophan; Surface =

alanine, serine, threonine, aspartic acid, asparagine, glutamine, glutamic acid, arginine, lysine and histidine; Boundary = alanine, serine, threonine, aspartic acid, asparagine, glutamine, glutamic acid, arginine, lysine, histidine, valine, isoleucine, leucine, phenylalanine, tyrosine, tryptophan, and methionine; Boundary XM = Boundary = alanine, serine, threonine, aspartic acid, asparagine, glutamine, glutamic acid, arginine, lysine, histidine, valine, isoleucine, leucine, phenylalanine, tyrosine, and tryptophan; All 20 = all 20 naturally occurring amino acids.

Please replace paragraph [190] with the following amended paragraph marked to show changes:

[190] Computational screening was applied to design energetically favorable interactions at the ~~Fc/Fc□R~~ Fc/FcγR interface at groups of variable positions that mediate or potentially mediate binding with ~~Fc□R~~ FcγR. Because the binding interface involves a large number of Fc residues on the two different chains, and because ~~Fc□Rs~~ FcγRs bind asymmetrically to Fc, residues were grouped in different sets of interacting variable positions, and designed in separate sets of calculations. In many cases these sets were chosen as groups of residues that were deemed to be coupled, that is the energy of one or more residues is dependent on the identity of one or more other residues. Various template structures were used, and in many cases calculations explored substitutions on both chains. For many of the variable position sets, calculations were carried out using both the PDA® and SPA™ technology computational screening methods described. The results of these calculations and relevant parameters and information are presented in Tables 1 – 30 below.

Please delete page 94.

Please replace description directly below Table 59 with the following amended description marked to show changes:

SPA™ technology; ~~Fc/Fc□RIIb~~ Fc/FcγRIIb model template structure; - carbohydrate

Please replace paragraph [206] with the following amended paragraph marked to show changes:

[206] In order to screen for Fc/FcγR binding, the extracellular regions of human V158 FcγRIIIa, human F158 FcγRIIIa, human FcγRIIb, human FcγRIIa, and mouse FcγRIII, were

expressed and purified. Figure 10 presents an SDS PAGE gel that shows the results of expression and purification of human V158 FcγRIIIa. The extracellular region of this receptor was obtained by PCR from a clone obtained from the Mammalian Gene Collection (MGC:22630). The receptor was fused with glutathione S-Transferase (GST) to enable screening. Tagged ~~Fc□RIIIa~~FcγRIIIa was transfected in 293T cells, and media containing secreted ~~Fc□RIIIa~~FcγRIIIa were harvested 3 days later and purified. For western analysis, membrane was probed with anti-GST antibody.

Please replace paragraph [207] with the following amended paragraph marked to show changes:

[207] Binding affinity to ~~Fc□RIIIa~~ FcγRIIIa and FcγRIIb was measured for all designed Fc variants using an AlphaScreen™ assay (Amplified Luminescent Proximity Homogeneous Assay (ALPHA), PerkinElmer, Wellesley, MA), a bead-based non-radioactive luminescent proximity assay. Laser excitation of a donor bead excites oxygen, which if sufficiently close to the acceptor bead generates a cascade of chemiluminescent events, ultimately leading to fluorescence emission at 520-620 nm. The AlphaScreen™ assay was applied as a competition assay for screening Fc variants. WT alemtuzumab antibody was biotinylated by standard methods for attachment to streptavidin donor beads, and GST-tagged FcγR was bound to glutathione chelate acceptor beads. In the absence of competing Fc variants, WT antibody and FcγR interact and produce a signal at 520-620 nm. Addition of untagged Fc variant competes with the WT Fc/FcγR interaction, reducing fluorescence quantitatively to enable determination of relative binding affinities. All Fc variants were screened for V158 FcγRIIIa binding using the AlphaScreen™ assay. Select Fc variants were subsequently screened for binding to FcγRIIb, as well as other FcγRs and Fc ligands.

Please replace paragraph [208] with the following amended paragraph marked to show changes:

[208] Figure 11 shows AlphaScreen™ data for binding to human V158 FcγRIIIa by select Fc variants. The binding data were normalized to the maximum and minimum luminescence signal provided by the baselines at low and high concentrations of competitor antibody respectively. The data were fit to a one site competition model using nonlinear regression, and these fits are represented by the curves in the figure. These fits provide the inhibitory concentration 50% (IC50) (i.e. the concentration required for 50% inhibition) for each

antibody, illustrated by the dotted lines in Figure 11, thus enabling the relative binding affinities of Fc variants to be quantitatively determined. Here, WT alemtuzumab has an IC50 of $(4.63x10^{-9})x(2) = 9.2$ nM, whereas S239D has an IC50 of $(3.98x10^{-10})x(2) = 0.8$ nM. Thus S239D alemtuzumab binds 9.2 nM / 0.8 nM = 11.64-fold more tightly than WT alemtuzumab to human V158 FcγRIIIa. Similar calculations were performed for the binding of all Fc variants to human V158 FcγRIIIa. Select Fc variants were also screened for binding to human ~~FcγRIIb~~FcγRIIb, and examples of these AlphaScreen™ binding data are shown in Figure 12. Table 61 presents the fold-enhancement or fold-reduction relative to the parent antibody for binding of Fc variants to human V158 FcγRIIIa (column 3) and human FcγRIIb (column 4), as determined by the AlphaScreen™ assay. For these data, a fold above 1 indicates an enhancement in binding affinity, and a fold below 1 indicates a reduction in binding affinity relative to WT Fc. All data were obtained in the context of alemtuzumab, except for those indicated with an asterix (*), which were tested in the context of trastuzumab.

Please replace paragraph [219] with the following amended paragraph marked to show changes:

[219] The SPR data corroborate the improvements to FcγRIIIa affinity observed by AlphaScreen™ assay. Table 62 further indicates the superiority of V264I/I332E and I332E over S298A and S298A/E333A/K334A; whereas S298A/E333A/K334A improves Fc binding to V158 and F158 FcγRIIIa by 1.7-fold and 4.7-fold respectively, I332E shows binding enhancements of 2.2-fold and 10.1-fold respectively, and V264I/I332E shows binding enhancements of 4.0-fold and 14-fold respectively. Also worth noting is that the affinity of V264I/I332E for F158 FcγRIIIa (52 nM) is better than that of WT for the V158 allotype (68 nM), suggesting that this Fc variant, as well as those with even greater improvements in binding, may enable the clinical efficacy of antibodies for the low responsive patient population to achieve that currently possible for high responders. The correlation between the SPR and AlphaScreen™ binding measurements are shown in Figures 23a – 23d. Figures 23a and 23b show the Kd - IC50 correlations for binding to V158 FcγRIIIa and F158 FcγRIIIa respectively, and Figures 23c and 23d show the fold-improvement correlations for binding to V158 FcγRIIIa and F158 FcγRIIIa respectively. The good fits of these data to straight lines ($r^2 = 0.9$, $r^2 = 0.84$, $r^2 = 0.98$, and $r^2 = 0.90$) support the accuracy the

AlphaScreen™ measurements, and validate its use for determining the relative ~~Fc□R~~Fcγ<u>R</u> binding affinities of Fc variants.

Please replace paragraph [226] with the following amended paragraph marked to show changes:

[226] Example 9. Protein A binding by Fc variants

As discussed, bacterial protein A binds to the Fc region between the ~~C□2~~ <u>Cγ2</u> and ~~C□3~~ <u>Cγ3</u> domains, and is frequently employed for antibody purification. The AlphaScreen™ assay was used to measure binding of select Fc variants to the protein A using biotinylated WT alemtuzumab antibody attached to streptavidin donor beads as described in Example 2, and using protein A coupled directly to acceptor beads. The binding data shown in Figure 28 for select Fc variants indicate that the capacity of the Fc variants to bind protein A is uncompromised. These results suggest that affinity of the Fc variants for other Fc ligands that bind the same site on Fc as protein A, such as the neonatal Fc receptor FcRn and protein G, are also unaffected.

Please replace paragraph [227] with the following amended paragraph marked to show changes:

[227] Example 10. Capacity of Fc variants to bind mouse ~~Fc□Rs~~ <u>Fcγ Rs</u>

Optimization of Fc to nonhuman ~~Fc□Rs~~ <u>Fcγ Rs</u> may be useful for experimentally testing Fc variants in animal models. For example, when tested in mice (for example nude mice, SCID mice, xenograft mice, and/or transgenic mice), antibodies and Fc fusions that comprise Fc variants that are optimized for one or more mouse ~~Fc□Rs~~<u>Fcγ Rs</u> may provide valuable information with regard to efficacy, mechanism of action, and the like. In order to evaluate whether the Fc variants of the present invention may be useful in such experiments, affinity of select Fc variants for mouse FcγRIII was measured using the AlphaScreen™ assay. The AlphaScreen™ assay was carried out using biotinylated WT alemtuzumab attached to streptavidin donor beads as described in Example 2, and GST-tagged mouse FcγRIII bound to glutathione chelate acceptor beads, expressed and purified as described in Example 2. These binding data are shown in Figure 29. Results show that some Fc variants that enhance binding to human FcγRIIIa also enhance binding to mouse FcγRIII. This result indicates that the Fc variants of the present invention, or other Fc variants that are optimized for nonhuman FcγRs, may find use in experiments that use animal models.